PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 31/4152, A61P 31/18 — A1

(11) International Publication Number: **WO 00/54771**

(43) International Publication Date: 21 September 2000 (21.09.00)

(21) International Application Number: PCT/NO00/00086

(22) International Filing Date: 10 March 2000 (10.03.00)

(30) Priority Data:
19991244 12 March 1999 (12.03.99) NO

(71) Applicant *(for all designated States except US)*: NORMEDICA AS [NO/NO]; Strandveien 56, N–1366 Lysaker (NO).

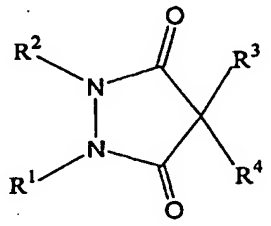
(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: KROGDAL, Toril, Garatun [NO/NO]; Mariesvei 17b, N–1322 Høvik (NO).

(74) Agent: OLSO PATENTKONTOR AS; Postboks 7007 M, N–0306 Oslo (NO).

(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), DM, DZ, EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (Utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims.

(54) Title: THE USE OF 3,5–PYRAZOLIDINEDIONE DERIVATIVES TO COMBAT VIRAL INFECTIONS



(I)

(57) Abstract

The invention relates to the use of a compound having formula (I), wherein R^1, R^2, R^3 and R^4 are as specified in the specification, preferably the compound is Oxyphenbutazone, and pharmaceutical salts thereof for preparing a pharmaceutical composition for inhibiting viral infections, especially HIV/AIDS and measles. Moreover, the invention concerns related methods of treating the same.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

THE USE OF 3,5-PYRAZOLIDINEDIONE DERIVATIVES TO COMBAT VIRAL INFECTIONS

TECHNICAL FIELD

This invention relates to specified substances and compositions thereof that reduce viral replication by interacting with infected cells including macrophages, monocytes and T cells from vertebrates, including mammals, particularly humans, protecting them from developing viral infection. This inhibition constitutes a therapy for viral diseases including HIV/AIDS and measles. More particularly, this invention relates to the new action of such compounds and compositions comprising such compounds and related methods of treatment for viral diseases. In one specific aspect, this invention pertains to methods for treating HIV/AIDS. In a second specific aspect, this invention pertains to the treatment of measles and other viral diseases (Goetz, O. And Peller, P., Klin. Wachr. 50, 751-753 (1972)). The definition of the class to be protected is the inclusion of the necessary submolecular group and derivatives thereof in any given member of the class to produce this inhibition.

BACKGROUND OF THE INVENTION

Development of antiviral drugs with direct viral targets (RT and protease inhibitors, integrase inhibitors under development) has so far resulted only in drugs severely exposed to resistance development (Pathogenesis of HIV/AIDS). As a result drugs with general cytotoxic mechanisms have been tested for specificity against viral infections (e.g., Thalidomide). The benefit of a reasonably specific cytotoxic substance is the lack of exposure to resistance development.

To replicate and produce progeny virus all viruses are strongly dependent on the metabolism and metabolizing machinery of the cells they infect. It is also well documented that all viruses interfere with the metabolism of the infected cell thereby modifying the cell in a number of different ways. For some virus/cell systems the ultimate outcome of an infection is cell death, for other systems cell transformations are observed and for other systems only minor changes of the infected cell can be detected.

Human immune deficiency virus (HIV) infects human cells including macrophages, monocytes and T-cells. Acquired immune deficiency syndrome (AIDS) results from infection with HIV.

AIDS is characterized by extensive immunosuppression that predisposes patients to life-threatening opportunistic infections as well as unusual forms of neoplasm. As to the other known subgroups or types of human T-lymphotropic viruses, Type I (HTLV-I) is believed to cause T-cell proliferation in leukemia. The role of HTLV-II in pathogenesis remains unclear, although it has been associated with rare cases of the T-cell variant of hairy cell leukemia (Golde et al. (1986), Seminars in Hematol. 23:3-9).

Synthesis of DNA complementary to viral RNA is required for both retroviral integration into host DNA and for the generation of new virions. For this reason, the HIV-encoded reverse transcriptase is a logical target for the development of agents for the treatment of patients with the acquired immunodeficiency syndrome (De Clercq et al. (1986) J. Med. Chem., 29:1561-1569), and with other diseases of retroviral origin.

Mitsuya et al. ((1985) Proc. Natl. Acad. Sci. USA, 82:7006-7100) reported that 3'-azido-3'-deoxythymidine (AZT) blocked the replication of HIV in cultured human T lymphoblasts, and inhibited the cytopathic effects of the virus. AZT was presumably phosphorylated by the T-cells and converted to the 5'-triphosphate derivative. That derivative was reported by those authors to be an inhibitor of HIV reverse transcriptase activity. Yarchoan et al. ((1986) Lancet, i:575-580), administered AZT to patients with AIDS or AIDS-related disease complexes. The drug was reportedly well tolerated and crossed the blood/brain barrier.

Observations made in clinical trials with AZT have shown that treatment of patients with AIDS or AIDS-related complex with AZT has resulted in elevation of CD4 (T4) peripheral blood cell counts, restoration of cutaneous delayed hypersensitivity, and reduction of the rate of opportunistic infections and death; results that can be related to the effect of AZT on T-cells.

From EP 0 237 796 A3 it has previously been suggested to use a prostaglandin synthesis inhibitor for manufacturing an anti-AIDS medicament, the relevance of this document to the present invention being that the compound oxyphenbutazone is mentioned among several others to be used in this manner. However, no actual data relating to the effect of oxyphenbutazone is given therein. This information concerning oxyphenbutazone has then to be considered on the basis of a subsequent patent application US Patent 4.956.377.

The observations referred in US Patent 4,956,377 provided an indication that a therapeutic effect against HIV might

be possible. The patent describes a specific substance and specific mechanics, both of which were abandoned after conducting formal studies leading the person skilled in the art to presume that this was a non-viable line of pharmaceuticals for viral diseases.

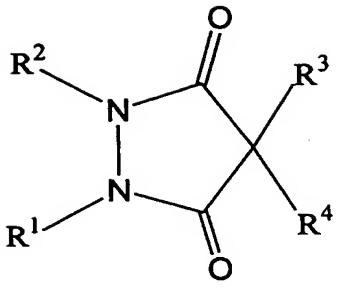
The present discovery that this class of substances will reduce replication of infected cells can be used to inhibit viral disease in vertebrates, including mammals, particularly human HIV/AIDS and measles, and has proven to possess a better inhibitory effect than AZT.

SUMMARY OF THE INVENTION

The present invention contemplates the use of a class of compounds, and compositions thereof, for treating viral diseases including HIV/AIDS and measles by reducing metabolic activity in cells infected with HIV/AIDS, measles and other viral diseases thereby reducing viral production and potentially killing the infected cell. The present invention also pertains to methods for treating viral infections, including HIV/AIDS and measles as well as combination therapies.

DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns the use of compounds having formula (I):



or therapeutic acceptable salts thereof,

wherein

R^1 and R^2, which are similar or different, are chosen from the group comprising

hydrogen;

alkyl;

functionalised alkyl;

phenyl;

benzoyl which may be substituted in any position with one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;

pyridinyl which may be attached to the molecular core in position 2, 3 or 4 and which may be substituted in any position, with the exception of position 1, with one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;

2-thiazolyl which may be substituted in the 4 and 5 positions with one or two of the groups alkyl and phenyl;

1,2-benzisothiazol-2(3H)-yl;

1,2-benzisothiazol-2(3H)-yl S,S-dioxide;

3-oxo-1,2-benzisothiazol-2(3H)-yl S,S dioxide;

1H-1,2,4-triazol-3-yl which may be substituted in position 5 with one of the groups alkyl and phenyl;

R^3 and R^4, being similar or different, are chosen from the group comprising

hydrogen;
hydroxy;
halogen;
alkyl;
functionalised alkyl;
hydroxyalkyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenylamino and diphenylamino wherein the phenyl groups may be similar or different;
aminoalkyl, (alkylamino)alkyl, (dialkylamino)alkyl, (phenylamino)alkyl and (diphenylamino)alkyl wherein the alkyl groups may be similar or different, and wherein the phenyl groups may be similar or different;
(4-methylmorpholinyl)alkyl;
benzoyl which may be substituted in any position with one ore more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2,3 or 4 and which may be substituted in any position, except for position 1, by one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;
alkanoyl;
oxoalkyl which may be substituted in any position with alkyl or phenyl;
phenylazo;
alkenyl which may be completely or partially substituted with deuterium, halogen, nitro, phenylthio, phenylsulphinyl and phenylsulphonyl;
2,3-dihydro-1,3-dioxo-1H-indene-2-yl which may be substituted in one or more positions with one or more of the groups alkyl, acyloxy, hydroxy and halogen;
1,3-dihydro-3-oxo-1-isobenzofuranyl;
phenyl(phenylimino)methyl which may be completely or partially deutetrated in any position, or substituted in

one or more positions with one or more of the groups alkoxy, alkanoyl, alkyl, halogen, hydroxy and nitro;
phenyl;
2-furfuryl;
1H-indol-3-yl;
(1H-indol-3-yl)methyl;

and wherein R^3, R^4 is the methylidene group $=C(R^5)R^6$ wherein R^5 and R^6 are similar or different and are chosen form the group comprising
hydrogen;
hydroxy;
alkyl which may be completely or partially substituted with halogen, preferably fluorine and chlorine;
alkenyl;
phenyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenymethyleneamin;
2-furanyl;
1H-indol-2-yl;
1H-indol-3-yl;

or are
2,5-bis[(3-ethyl-2(3H)-benzothiazolylidene)ethylidene]-cyclopentyliden;
cycloalkylidene with 4 or more C-atoms in the ring;
diazo;
4-[(4-chlorophenyl)methylene]-5-thioxo-3-isothiazol-idinylidene;
1,5-dihydro-5-oxo-2H-pyrrol-2-ylidene which may be substituted in position 1 with one of the groups phenyl and/or in position 5 with the groups phenylhydrazono or phenylimino;
dihydro-5-oxo-2(3H)-furanylidene:

3,5-dioxo-4-pyrazolidinylidene;
4(1H)-quinolinylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(3-ethyl-2(3H)-benzothiazolylidene)methyl]ethylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(1-ethyl-3,4-dihydro-2(1H)-quinolinylidene)methyl]ethylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(1-ethyl-2(1H)-quinolinylidene)methyl]ethylidene;
(3-ethyl-2-oxazolidinylidene)ethylidene,

as well as compositions comprising them, for inhibiting cellular functions to combate viral production.

The term "C_{1-6}alkyl" as used herein, means a C_{1-6}alkyl which may be straight, branched or cyclic, optionally substituted with phenyl.

The term "functionalised C_{1-6}alkyl" as used herein, means a C_{1-6}alkyl which may be completely or partially substituted with deuterium, halogen, phenyl, alkanoyl, hydroxy, nitro, 1,3-dioxolan-2-yl, alkylthio, phenylthio, phenylsulphinyl and phenylsulphonyl, 2-oxiranyl, 3-alkyloxiranyl and 3,3-dialkyloxiranyl, 2-furfuryl and 4-oxo-4H-1-benzopyran-3-yl.

The term "phenyl" as used herein, means phenyl and phenyl which may be completely or partially deuterated in any position, or substituted in one or more positions by one or more of the groups alkyl, alkoxy including 2-methoxy-ethoxymethoxy, phenoxy, alkanoyl, acyloxy, halogen, hydroxy, nitro and nitroso;

The term "hydroxyalkyl" as used herein, means C_{1-6}alkyl as defined supra and wherein one or more hydroxy groups are attached to any position on the alkyl.

The term "alkanoyl" as used herein, means C_{1-6}alkanoyl wherein the alkyl group attached to the carbonyl group is substituted with hydrogen, alkyl, alkylamino, dialkylamino, phenylamino and diphenylamino, or with pyrrolidinyl and 2,6-dimethyl-1-piperidinyl;

The term "oxoalkyl" as used herein, means C_{1-6}oxoalkyl wherein alkyl is as defined supera.

The term "alkenyl" as used herein, means C_{2-6}alkenyl which may be straight, branched or cyclic and optionally substituted with one or more of the groups amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different.

The term "alkoxy" as used herein, means C_{1-6}alkoxy wherein the alkyl group being attached to the oxygen is as defined supra.

The term "acyloxy" as used herein, means C_{1-6}acyloxy wherein the alkanoyl group being attached to the oxygen is as defined supra.

The term "therapeutic salt" as used herein, also comprises the solvates that the compounds of formula (I) as well as the salts thereof, are able to form. Such solvates are for example hydrates, alcholates an the like.

More preferred compounds having formula (I) or therapeutic acceptable salts thereof are compounds wherein:

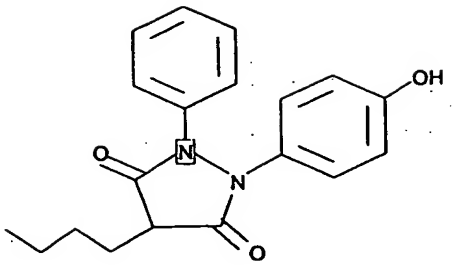
R^1 and R^2, which are similar of different, are chosen from the group comprising
hydrogen;
alkyl;

functionalised alkyl;
phenyl;
benzoyl which may be substituted in any position with one or more of the groups alkoxy, alkyl, fluorine, chlorine, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2, 3 or 4 and which may be substituted in position 5 with one of the groups fluorine and nitro;
2-thiazolyl which may be substituted in the 4 and/or 5 positions with one or two of the groups alkyl and phenyl;
R^3 and R^4, being similar or different, are chosen from the group comprising
hydrogen;
hydroxy;
fluorine;
alkyl;
functionalised alkyl;
hydroxyalkyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenylamino and diphenylamino wherein the phenyl groups may be similar or different;
aminoalkyl, (alkylamino)alkyl, (dialkylamino)alkyl, (phenylamino)alkyl and (diphenylamino)alkyl wherein the alkyl groups may be similar or different, and the phenyl groups may be similar or different;
(4-methylmorpholinyl)alkyl;
benzoyl which may be substituted in any position with one ore more of the groups alkoxy, alkyl, fluorine, chlorine, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2,3 or 4 and which may be substituted in position 5 with fluorine, chlorine, hydroxy or nitro;
alkanoyl;

oxoalkyl which may be substituted in any position with alkyl or phenyl;
phenylazo;
alkenyl;
phenyl(phenylimino)methyl;
phenyl;
2-furfuryl;
1H-indol-3-yl;
(1H-indol-3-yl)methyl;

and wherein R^3, R^4 is the methylidene group $=C(R^5)R^6$ wherein R^5 and R^6 being similar or different, are chosen form the group comprising
hydrogen;
hydroxy;
alkyl which may be completely or partially substituted with fluorine and chlorine;
alkenyl which may be substituted with one or more phenyl groups, and which alkenyl may be completely or partially substituted with deuterium, fluorine, chlorine, nitro, phenylthio, phenylsulphinyl and phenylsulphonyl;
phenyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenymethyleneamin;
2-furanyl;
1H-indol-2-yl;
1H-indol-3-yl.

The compound named Oxyphenbutazone, i.e., 4-butyl-1-(4-hydroxyphenyl)-2-phenyl-3,5-pyrazolidinedione, having formula (II)



(I)

represents an especially preferred embodiment of the present invention.

It is to be understood that the successful application of derivatives within the indicated group of these compounds are used to inhibit viral diseases as described herein was quite unexpected.

It has been known for some time that NSAIDs (non-steroid anti-inflammatory drugs), particularly antiphlogistic compounds, previously used to treat rheumatoid disorders might have an effect on viral disease (Steinmeyer&Kalbhen, Inflamm. Res. 1996 Jul;45(7):324-9). However, due to the great number of compounds in this group and lack of understanding of the underlying mechanism it has prior to the present invention not been possible to identify any specific compound with sufficient therapeutic effect.

Non-steroid anti-inflammatory compounds used against rheumatoid disorders were previously thought to inhibit the synthesis of prostagladines. However, their method of action is still unclear.

The results of the laboratory studies described in detail below show that Oxyphenbutazone reduces the HIV virus count more than AZT, a well known therapy for HIV/AIDS. The

results presented are not intended to limit the scope of the invention in any way.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows the cytotoxic effects of AZT.

Figure 2 shows the cytotoxic effect of AZT after 4 days of incubation.

Figure 3 shows the cytotoxic effect of Oxyphenbutazone (oxyPB).

Figure 4 shows the cytotoxic effect of oxyPB after 4 days of incubation.

Figure 5 shows the effect of AZT on viral replication.

Figure 6 shows the effext of oxyPB on viral replication.

Figure 7 shows the effect of OxyPB on Taq Polymerase.

EXAMPLES

Example 1

The aims of this study were to evaluate the cytotoxic effects and the antiviral effects with respect to inhibition of HIV growth *in vitro* in tissue cultures. The effect of the compounds on cell growth and virus production was compared with AZT.

Cultivation of cells

In this study the two human CD4' lymphocyte cell lines Sup TI and Jurkat were applied. Sup TI is derived from a Non-Hodgkin's T-cell lymphoma patient (Smith SD *et al* (1984), Cancer Research, 44, 5657) and was a gift from Dr. J. Sodroski at the Division of Human Retroviruses, Dana Farber Cancer Institute, Harvard Medical School, Boston, US. The Jurkat cell line has a similar origin and was obtained from the American Tissue Culture Collection (ATCC). These cell lines were chosen for the studies due to their high content of CD4' receptors and ability to form large syncytia

following infection with HIV-1. The cells were cultivated as suspensjon cultures in plastic flasks (Nunc) in RPMI 1640 medium, (Bio Whittaker) supplemented with 5% Foetal Calf Serum, 2 mM glutamine (both from Bio Whittaker) and ABAM (Anti Biotic Anti Mycotic) in 1 mM final concentrations (Sigma Chem. Company). The Anti Biotic Anti Mycotic solution consists of penicillin and fungizone. Gentamicine (Bio Whittaker) is added to a final concentration of 50 µg/ml.

Counting of cell numbers was performed the same day the experiments started using the Trypan blue exclusion method and a Burker counting chamber at a magnification of 400X. The ratio between live and dead cells was at least 95/5 in all experiments and prior to the experiments the medium was half-changed. The cell density was adjusted to approximately $1x10^6$ cells/ml and kept at this concentration throughout the experiments.

Testing of cytotoxic effects

The MTT assay method for determining the number of viable cells.

The principle of this assay is based on the cleavage of the yellow tetrazolium salt MMT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazoliumbromide (Thiazolyl blue) (Sigma Chemical Company)to form purple forniazan crystals due to the dehydrogenase activity in active mitochondria present in living cells (Mosman, T *et al* (1983) J. Immunol. Methods, 65, *55)*.

Standard curve for the MTT assay is established by diluting exponentially growing SupTI cells at known cell numbers in standard medium into 96 wells tissue culture plates (Nunc) at a total volume of 100 µl followed by adding 50 µl of MTT reagent (3 mg/ml in PBS) to each well. After addition of

the MTT reagent, the plates are incubated at 37°C and 5% CO_2 for 3 hours. Then the cells are centrifuged at 2000 rpm (800x g) for 10 minutes in a centrifuge equipped with micro-titer plate holders. After centrifugation 100 µl supernatant is removed from the wells. For this purpose a multi-channel micro-pipette is used.

The pelleted cells are resuspended in 100 µl DMSO and the plates are shaken slowly for about 10 min at room temperature before the absorption is read in a Titertek Multiscan Plus MK II (ELISA reader) photometer equipped with a 580 nm light filter. A near linear relationship between the amount of cells and the intensity of staining was obtained.

Effect of test substances on the growth of uninfected SupT1 cells

For each substance tested 3 parallel experiments were done. Survival of cells was tested between 2 and 7 days, respectively, after starting treatment of the cells with the substances.

The cells were maintained in 96 wells micro-titer plate. To each well $1x10^4$ cells in 100 µl medium were added. To the suspension of cells was then added 10 µl of the test substance in water and as diluent RPMI 1640 medium was used. At the end of incubation with the compounds, survival of cells was measured by adding the MTT reagent and the samples were processed as described above.

Results from these experiments are presented for AZT in figure 1 and for the test compound Oxyphenbutazone in figure 3 as relative cell numbers (MTT-values) as a function of dose and time (days) of incubation. In figures 2 (AZT) and 4 (Oxyphenbutazone) are the effect of the

compounds on cell growth after four days of incubation shown as a function of concentration of the compounds. Almost similar curves would have been obtained using the other time points from figures 1 or 3. The main conclusion which can be drawn from these data is that both AZT(as expected from previously published reports) and Oxyphenbutazone affects the growth of these cells in culture. Furthermore it can be concluded that antiviral effects should not be studied at concentrations above 10μM since the effect on cell growth would directly influence the viral production.

The toxic doses of AZT and Oxyphenbutazone observed in these studies can not directly be related to toxic doses of these compounds *in vivo* since in tissue cultures the cells are directly exposed to the compounds while *in vivo* the picture is much more complex due to the heterogenic environment for the different cells.

If even distribution of AZT or Oxyphenbutazone is assumed in a human body of approximately 70 kg a concentration of 1 mM would have been obtained if 15 mg AZT or 18 mg Oxyphenbutazone had been given to the person.

Testing for anti-viral effects

The initial screening of anti-viral effects of the test compounds was based on measuring the formation of syncytia in relation to concentration of the compounds. For this purpose, cells were grown in micro-titer plates for fast screening and in T25 flasks (Nunc) for more accurate measurements.

Micro-titer plates are used to screen a large number of test-substances using $1x10^4$ cells/well. It is previously experienced that this technique is suitable when screening

the test-substances, but exact number of syncytia is difficult to obtain due to aggregation of cells in the small wells.

To estimate the exact number of syncytia present after infection of cells by HIV-1 we have found that T25 flasks (Nunc) are more convenient. HIV-1 containing supernatant from Molt 3 IIIB cell-supernatant is prepared by centrifuging the cells at 1000 rpm for 5 mill. The Molt 3 IIIB cell line is producing HIV virus particles constitutively. The cell line was established in our laboratory by infecting Molt 3 cells (American Type Culture Collections, ATCC CRL 1552,) with the HIV-1 strain HTLV IIIB obtained from Dr. W.A. Haseltine at the Division of Human Retroviruses, Dana Farber Cancer Institute, Harvard Medical School, Boston, US.

In order to standardise the supernatant with respect to the amount of virus, p24 Ag antigen was measured using an ELISA based technique.

Each virus supernatant to be used in the experiment should have a p24 Ag antigen concentration of 1.5 -2 ng/10^5 cells. Each flask was filled with $1x10^6$ cells/ml in a total volume of 5 ml. The test substances were added 30 minutes prior to the addition of the virus containing supernatant and during this preincubation time the flasks were kept at 37°C and 5% CO_2.

After preincubation 500 µl of virus supernatant is added, and the number of syncytia was counted after 24 and 48 hours incubation; this being the standard times for optimal syncytia formation for these cell lines at the concentration of virus used.

For each test-substance 2 flasks in parallel were used and the syncytia in each flask counted by counting the number of syncytia on 5 different places in the flask, thus giving 10 independent counts for each test-substance. The parallels obtained are usually, and should be, within 10% variation for each experiment.

The total amount of syncytia in infected cultures at different concentrations of test substances are given in tables and plotted in figures. In the graphs the results after 24 hours and 48 hours will be presented. Supernatants (0.5 ml) from the cell cultures were collected every day and were analysed with respect to HIV content measured as p24 antigen. For this purpose a commercially available ELISA was used. For the ELISA test the supernatants were centrifuged free of cells at low centrifugation forces.

Data from these studies are shown in figure 5 for AZT and in figure 6 for Oxyphenbutazone.

In figure 5 the amount of p24 antigen observed in the supernatants taken at different time points (days) are plotted against the concentration of AZT in the HIV infected cell cultures and in figure 6 the amount of p24 antigen observed in the supernatants taken at different time points (days) are plotted against the concentration of Oxyphenbutazone in the HIV infected cell cultures.

It is evident from the data presented in these two figures that both with AZT and with Oxyphenbutazone a concentration dependent reduction of virus production can be observed. From these experiments it can also be concluded that at the experimental conditions used Oxyphenbutazone is a better anti-viral agent than AZT.

The data presented show (indicate) that different cell types are sensitive to the specified substances at different concentrations of the drugs. An antiviral effect will therefore be explained by the fact that infected cells of the macrophage, monocyte or T-cell lineages are sensitised following infection and production of HIV and other cell types will be sensitised by other viruses infecting them.

The reduction of HIV production at certain concentrations of the test substances described herein and experimental conditions used as well as the effect of production of viral DNA as measured by PCR reactions using the Taq-polymerase and specially designed viral primer pairs all demonstrate reduction in viral replication. The data also indicate that the viral infection and replication turns the cell more vulnerable to the specified substances.

Example 2

The Effect of Oxyphenbutazone on Taq Polymerase

Background

The effect of Oxyphenbutazone on Taq Polymerase has been investigated. Taq Polymerase is a thermostable DNA polymerase isolated from *Thermus Aquaticus*. Taq Polymerase is stable at temperatures up to 95°C and displays the highest activity at 72°C.

The PCR involves a repeated series of temperature variations corresponding to denaturation of the template, annealing of the primers and the final extension step. The effect of the high and rapid variations in temperature on

the compound is not known. This must be taken into consideration when judging the results.

Procedure

A previously well-established reaction was modified as to enable it to screen for inhibition of Taq Polymerase. The pSVC21 plasmid containing the full length HIV-1 genome (HxB2) was used as a template and primers spanning the RT encoding region in the *Pol* gene were used to amplify the region encoding HIV-1 RT. The amplified region corresponds to a 1681 bp DNA fragment easily detected on an agarose gel.

Primers RT5` 5- GGGAATTCCCCATTAGCCCTATTGG-3
RT3` 5- CCGGGATCCTAGTACTTTCCTGATTC-3

The following reactions were set up:

5 µl 10x PCR buffer (Geneamp Perkin Elmer)
3 µl 15 µM RT5`Primer
3 µl 15 µM RT3`Primer
5 µl dNTP (2.5 mM each of dATP, dGTP, dCTP, dTTP. Promega U1240)
1 µl Taq Polymerase (1U/µl) (Perkin Elmer AmpliTaq N808-0155)
1 µl ~0.5 ng/ µl pSVC21
7 µl ddH_2O
25 µl Oxyphenbutazone in 2x final concentration
=50 µl total volume

A 24-sample reaction-mixture was prepared (12 µl 10x PCR buffer, 72 µl RT5`, 72 µl RT3`, 120 µl dNTP, 24 µl Taq

Polymerase, 168 µl ddH_2O, 24 µl pSVC21). Twenty-five µl of this mixture was transferred to a PCR tube already containing the 25 µl of the compound. The reaction mixture was briefly heated to room temperature when added to the compound to avoid precipitation.

The following program was run:

Start	35 cycles			End
94°C	94°C	50°C	72°C	72°C
4 minutes	30 seconds	45 seconds	3 minutes	7 minutes

The reaction was run on a Perkin Elmer 2400 thermocycler.

The PBS, used for dissolving the compound, inhibited the PCR almost completely and this necessitated a new method for dissolving the compound. A 100 mM concentration of the compound was prepared in 100% ethanol. It was attempted to further dilute the compound down to 5 mM ddH_2O. The compound did precipitate strongly at this concentration. The compound dissolves readily in PBS at this concentration At 1 mM the compound seemed to be soluble. The concentration of EtOH at this concentration of the compound is 1%.

Amplification of HIV-1 RT was purely coincidental and has nothing to do with the proposed application of the compound.

Results

There seems to be a correlation between increasing concentrations of the compound and inhibition of the PCR. The concentration of ethanol at 500 µM of the compound is

0.5%. This did not inhibit the reaction, suggesting that the compound causes the effect.

The experiments were repeated and the results were reproducible. There were some differences in the amount of inhibition, especially at lower concentrations of the compound. This is believed to be a result of the way the compound is prepared (dissolving it in water; greater instability concerning solubility of the compound) and the semi quantitative nature of the experiment.

Figure 7 shows the product of PCR analysed on an agarose gel wherein two parallels of the reactions were run. The numbers refer to both the upper and lower wells of the gel. The size of the band (in bp) corresponds to what is being expected. The digits refer to

1. 1pGEM
1. Positive control (no compound X, only water)
2. 5 µm X
3. 10 µm X
4. 20 µm X
5. 50 µm X
6. 100 µm X
7. 150 µm X
8. 200 µm X
9. 300 µm X
10. 500 µm X
11. Control containing only the solvent equal to the 500 µm sample
12. Control containing only the solvent equal to the 500 µm sample

Example 3
Composition

A tablet including the active substance according to the present invention, Oxyphenbutazone, may be formulated as follows:

100 mg Oxyphenbutazone mixed with 100 mg aluminium hydroxide and 100 mg magnesium trisilicate.

Treatment of virally infected animals and humans with the specific substances will lead to a reduction in viral titers and thereby improving combating the viral infection by the immune system. Furthermore, a reduction in virus production by the specified substances will reduce the production of mutated viruses giving rise to drug resistance when the patient (or host) is undergoing antiviral treatment by drugs (e.g. AZT) directly interacting with the viral replication machinery.

Antiviral drugs may have several ways of interacting with the recipient cells, e.g. by blocking the entry of the virus into the cell (blocking receptors, camouflaging the cell from the virus etc), by interfering with the virus' replication on an intra-cellular level (inhibiting enzymes crucial for viral replication, inhibiting integration or autonomal replication of viral DNA inside the cell, inhibiting the assembly of viral progeny etc.) or by interfering with the liberation of viruses from the infected cells. It will also be possible to combat viral infection by making the infected cells more susceptible to drugs killing them or making such cells suicidal.

Initial studies of the compounds according to the present invention indicate that the compounds have an intra-cellular action interfering with the production of viruses inside the infected cells. The exact action of the compounds is at present uncertain, but its effect is at least as pronounced as AZT being a much used drug in anti-retroviral therapy.

The invention further relates to the use of the compounds having formula (I), and especially Oxyphenbutazone, according to the invention together with pharmacological

adjuvants and vehicles that are physiologically compatible and not detrimental for the action of the compounds in pharmaceutical compositions. Such substances are described in Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. (1980).

For this therapeutic effect, the suitable dose differs and depends, for example, on the host, the type of administration, and the type and severity of the conditions to be treated, but in general, satisfactory results are to be expected in vertebrate with daily doses of 0.1 to 3000 mg/kg of body weight. With large mammals, e.g., humans, there is a recommended daily dose of 0.1 to 3000 mg of the compounds according to the invention. Preferred are values of 1 to 500 mg per day, and most preferred is 2 to 30 mg per day. 200 mg of Oxyphenbutazone is equal to 15 µmol of the compound. The daily dose of the compositions including the compounds according to the invention should be administered in 1 to 3 partial doses per day.

The compounds according to the invention can be administered by any usual method in the case of systemic treatment, especially enterally, preferably parenterally, such as subcutaneously, intravenously, intradermally and orally, and most preferably orally. Tablets, capsules, drops, suppositories, injection solutions, or suspensions are the appropriate forms for administration.

Combination therapies are also contemplated by the invention wherein a compound according to the invention having formula (I) or (II) is used for preparing a composition including additionally an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor for treating viral infections, especially HIV/AIDS and measles.

The invention further relates to a method of treating a viral infection, especially HIV/AIDS and measles, wherein a compound according to the invention having formula (I) or (II) is administered to a subject in need of such a treatment.

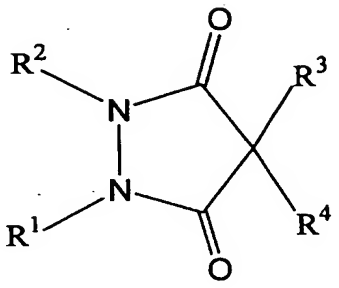
A method of treating a viral infection comprises to administer a compound or pharmaceutical composition according to the present invention systemically, especially enterally, preferably parenterally, such as subcutaneously, intravenously, intradermally and orally, and most preferably orally.

A method of treating a viral infection does also include to administer a compound according to the invention together with an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor for treating viral infections, especially HIV/AIDS and measles.

Claims

What is claimed is:

1. Use of a compound having formula (I):



or therapeutic acceptable salts thereof,

wherein

R^1 and R^2, which are similar or different, are chosen from the group comprising

hydrogen;

alkyl;

functionalised alkyl;

phenyl;

benzoyl which may be substituted in any position with one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;

pyridinyl which may be attached to the molecular core in position 2, 3 or 4 and which may be substituted in any position, with the exception of position 1, with one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;

2-thiazolyl which may be substituted in the 4 and 5 positions with one or two of the groups alkyl and phenyl;

1,2-benzisothiazol-2(3H)-yl;

1,2-benzisothiazol-2(3H)-yl S,S-dioxide;
3-oxo-1,2-benzisothiazol-2(3H)-yl S,S dioxide;
1H-1,2,4-triazol-3-yl which may be substituted in position 5 with one of the groups alkyl and phenyl;

R^3 and R^4, being similar or different, are chosen from the group comprising
hydrogen;
hydroxy;
halogen;
alkyl;
functionalised alkyl;
hydroxyalkyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenylamino and diphenylamino wherein the phenyl groups may be similar or different;
aminoalkyl, (alkylamino)alkyl, (dialkylamino)alkyl, (phenylamino)alkyl and (diphenylamino)alkyl wherein the alkyl groups may be similar or different, and wherein the phenyl groups may be similar or different;
(4-methylmorpholinyl)alkyl;
benzoyl which may be substituted in any position with one ore more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2,3 or 4 and which may be substituted in any position, except for position 1, by one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;
alkanoyl;
oxoalkyl which may be substituted in any position with alkyl or phenyl;
phenylazo;

alkenyl which may be completely or partially substituted with deuterium, halogen, nitro, phenylthio, phenylsulphinyl and phenylsulphonyl;
2,3-dihydro-1,3-dioxo-1H-indene-2-yl which may be substituted in one or more positions with one or more of the groups alkyl, acyloxy, hydroxy and halogen;
1,3-dihydro-3-oxo-1-isobenzofuranyl;
phenyl(phenylimino)methyl which may be completely or partially deutetrated in any position, or substituted in one or more positions with one or more of the groups alkoxy, alkanoyl, alkyl, halogen, hydroxy and nitro;
phenyl;
2-furfuryl;
1H-indol-3-yl;
(1H-indol-3-yl)methyl;

and wherein R^3, R^4 is the methylidene group $=C(R^5)R^6$ wherein R^5 and R^6 are similar or different and are chosen form the group comprising
hydrogen;
hydroxy;
alkyl which may be completely or partially substituted with halogen, preferably fluorine and chlorine;
alkenyl;
phenyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenymethyleneamin;
2-furanyl;
1H-indol-2-yl;
1H-indol-3-yl;

or are
2,5-bis[(3-ethyl-2(3H)-benzothiazolylidene)ethylidene]-cyclopentyliden;

cycloalkylidene with 4 or more C-atoms in the ring;
diazo;
4-[(4-chlorophenyl)methylene]-5-thioxo-3-isothiazolidinylidene;
1,5-dihydro-5-oxo-2H-pyrrol-2-ylidene which may be substituted in position 1 with one of the groups phenyl and/or in position 5 with the groups phenylhydrazono or phenylimino;
dihydro-5-oxo-2(3H)-furanylidene:
3,5-dioxo-4-pyrazolidinylidene;
4(1H)-quinolinylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(3-ethyl-2(3H)-benzothiazolylidene)methyl]ethylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(1-ethyl-3,4-dihydro-2(1H)-quinolinylidene)methyl]ethylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(1-ethyl-2(1H)-quinolinylidene)methyl]ethylidene;
(3-ethyl-2-oxazolidinylidene)ethylidene,

for preparing a pharmaceutical composition for treating viral diseases/infections.

2. Use of a compound according to claim 1
wherein
R^1 and R^2, which are similar of different, are chosen from the group comprising
hydrogen;
alkyl;
functionalised alkyl;
phenyl;
benzoyl which may be substituted in any position with one or more of the groups alkoxy, alkyl, fluorine, chlorine, hydroxy and nitro;

pyridinyl which may be attached to the molecular core in position 2, 3 or 4 and which may be substituted in position 5 with one of the groups fluorine and nitro;
2-thiazolyl which may be substituted in the 4 and/or 5 positions with one or two of the groups alkyl and phenyl;
R^3 and R^4, being similar or different, are chosen from the group comprising
hydrogen;
hydroxy;
fluorine;
alkyl;
functionalised alkyl;
hydroxyalkyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenylamino and diphenylamino wherein the phenyl groups may be similar or different;
aminoalkyl, (alkylamino)alkyl, (dialkylamino)alkyl, (phenylamino)alkyl and (diphenylamino)alkyl wherein the alkyl groups may be similar or different, and the phenyl groups may be similar or different;
(4-methylmorpholinyl)alkyl;
benzoyl which may be substituted in any position with one ore more of the groups alkoxy, alkyl, fluorine, chlorine, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2,3 or 4 and which may be substituted in position 5 with fluorine, chlorine, hydroxy or nitro;
alkanoyl;
oxoalkyl which may be substituted in any position with alkyl or phenyl;
phenylazo;
alkenyl;
phenyl(phenylimino)methyl;
phenyl;

2-furfuryl;
1H-indol-3-yl;
(1H-indol-3-yl)methyl;

and wherein R^3, R^4 is the methylidene group $=C(R^5)R^6$ wherein R^5 and R^6 being similar or different, are chosen form the group comprising
hydrogen;
hydroxy;
alkyl which may be completely or partially substituted with fluorine and chlorine;
alkenyl which may be substituted with one or more phenyl groups, and which alkenyl may be completely or partially substituted with deuterium, fluorine, chlorine, nitro, phenylthio, phenylsulphinyl and phenylsulphonyl;
phenyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenymethyleneamin;
2-furanyl;
1H-indol-2-yl;
1H-indol-3-yl.

3. Use of a compound according to claim 1 for preparing a composition for treating HIV/AIDS.

4. Use of a compound according to claim 2 for preparing a composition for treating HIV/AIDS.

5. Use of a compound according to claim 1 for preparing a composition for treating measles.

6. Use of a compound according to claim 2 for preparing a composition for treating measles.

7. Use of a compound according to claim 1 wherein the compound is Oxyphenbutazone.

8. Use of Oxyphenbutazone for preparing a composition for treating HIV/AIDS.

9. Use of Oxyphenbutazone for preparing a composition for treating measles.

10. Use of a compound according to claim 7 for preparing a composition wherein Oxyphenbutazone is present in the composition in an amount suitable for delivering an effective dose of Oxyphenbutazone.

11. Use of a compound according to claim 10, wherein Oxyphenbutazone is present in the range of 0.0075 - 225 µmol/kg body weight.

12. Use of a compound according to claim 10, wherein Oxyphenbutazone is present in the range of 0.075 - 37.5 µmol/kg body weight.

13. Use of a compound according to claim 10, wherein Oxyphenbutazone is present in the range of 0.15 - 2.25 µmol/kg body weight.

14. Use of a compound according to claim 10 for preparing a composition suitable for being administered in 1 - 3 partial doses per day.

15. Use of a compound according to claim 1 for preparing a composition suitable for being administered systemically, especially enterally, preferably parenterally, such as subcutananeously, intravenously, intradermally and orally, most preferably orally.

16. Use of a compound according to claim 10 for making a composition suitable for being administered systemically, especially enterally, preferably parenterally, such as subcutananeously, intravenously, intradermally and orally, most preferably orally.

17. Use of a compound according to claim 1 for preparing a composition including additionally an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor.

18. Use of a compound according to claim 10 for preparing a composition including additionally an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor.

19. A method of treating a viral infection wherein a compound according to claim 1 is administered to a subject in need of such a treatment.

20. A method according to claim 19 for treating HIV/AIDS.

21. A method according to claim 19 for treating measles.

22. A method according to claim 19 for treating a viral infection wherein a compound according to claim 1 is administered orally to a subject in need of such a treatment.

23. A method according to claim 20 for treating HIV/AIDS wherein a compound according to claim 1 is administered orally to a subject in need of such a treatment.

24. A method according to claim 21 for treating measles wherein a compound according to claim 1 is administered orally to a subject in need of such a treatment.

25. A method of treating a viral infection wherein a compound according to claim 10 is administered to a subject in need of such a treatment.

26. A method according to claim 25 for treating HIV/AIDS.

27. A method according to claim 25 for treating measles.

28. A method according to claim 25 for treating a viral infection wherein a compound according to claim 10 is administered orally to a subject in need of such a treatment.

29. A method according to claim 26 for treating HIV/AIDS wherein a compound according to claim 10 is administered orally to a subject in need of such a treatment.

30. A method according to claim 27 for treating measles wherein a compound according to claim 10 is administered orally to a subject in need of such a treatment.

31. A method according to claim 19 wherein a compound according to claim 1 in addition to an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor is aministered.

32. A method according to claim 25 wherein a compound according to claim 10 in addition to an inhibitor for inhibiting a reverse transcriptase enzyme and/or

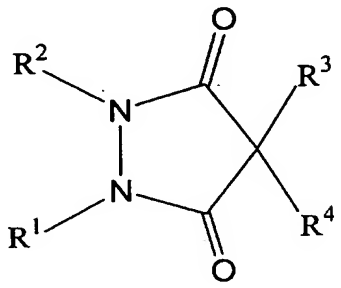
protease inhibitor and/or an integrase inhibitor is aministered.

AMENDED CLAIMS

[received by the International Bureau on 11 August 2000 (11.08.00) ;
original claims 1-32 replaced by amended claims 1-32 (10 pages)]

What is claimed is:

1. Use of a compound having formula (I):



or therapeutic acceptable salts thereof,

wherein

R^1 and R^2, which are similar or different, are chosen from the group comprising

hydrogen;

alkyl;

functionalised alkyl;

phenyl;

benzoyl which may be substituted in any position with one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;

pyridinyl which may be attached to the molecular core in position 2, 3 or 4 and which may be substituted in any position, with the exception of position 1, with one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;

2-thiazolyl which may be substituted in the 4 and 5 positions with one or two of the groups alkyl and phenyl;

1,2-benzisothiazol-2(3H)-yl;

1,2-benzisothiazol-2(3H)-yl S,S-dioxide;
3-oxo-1,2-benzisothiazol-2(3H)-yl S,S dioxide;
1H-1,2,4-triazol-3-yl which may be substituted in position 5 with one of the groups alkyl and phenyl;

R^3 and R^4, being similar or different, are chosen from the group comprising
hydrogen;
hydroxy;
halogen;
alkyl;
functionalised alkyl;
hydroxyalkyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenylamino and diphenylamino wherein the phenyl groups may be similar or different;
aminoalkyl, (alkylamino)alkyl, (dialkylamino)alkyl, (phenylamino)alkyl and (diphenylamino)alkyl wherein the alkyl groups may be similar or different, and wherein the phenyl groups may be similar or different;
(4-methylmorpholinyl)alkyl;
benzoyl which may be substituted in any position with one ore more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2,3 or 4 and which may be substituted in any position, except for position 1, by one or more of the groups alkoxy, alkyl, halogen, hydroxy and nitro;
alkanoyl;
oxoalkyl which may be substituted in any position with alkyl or phenyl;
phenylazo;

alkenyl which may be completely or partially substituted with deuterium, halogen, nitro, phenylthio, phenylsulphinyl and phenylsulphonyl;
2,3-dihydro-1,3-dioxo-1H-indene-2-yl which may be substituted in one or more positions with one or more of the groups alkyl, acyloxy, hydroxy and halogen;
1,3-dihydro-3-oxo-1-isobenzofuranyl;
phenyl(phenylimino)methyl which may be completely or partially deutetrated in any position, or substituted in one or more positions with one or more of the groups alkoxy, alkanoyl, alkyl, halogen, hydroxy and nitro;
phenyl;
2-furfuryl;
1H-indol-3-yl;
(1H-indol-3-yl)methyl;

and wherein R^3, R^4 is the methylidene group $=C(R^5)R^6$ wherein R^5 and R^6 are similar or different and are chosen form the group comprising
hydrogen;
hydroxy;
alkyl which may be completely or partially substituted with halogen, preferably fluorine and chlorine;
alkenyl;
phenyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenymethyleneamin;
2-furanyl;
1H-indol-2-yl;
1H-indol-3-yl;

or are
2,5-bis[(3-ethyl-2(3H)-benzothiazolylidene)ethylidene]-cyclopentyliden;

cycloalkylidene with 4 or more C-atoms in the ring;
diazo;
4-[(4-chlorophenyl)methylene]-5-thioxo-3-isothiazolidinylidene;
1,5-dihydro-5-oxo-2H-pyrrol-2-ylidene which may be substituted in position 1 with one of the groups phenyl and/or in position 5 with the groups phenylhydrazono or phenylimino;
dihydro-5-oxo-2(3H)-furanylidene:
3,5-dioxo-4-pyrazolidinylidene;
4(1H)-quinolinylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(3-ethyl-2(3H)-benzothiazolylidene)methyl]ethylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(1-ethyl-3,4-dihydro-2(1H)-quinolinylidene)methyl]ethylidene;
2-(3-ethyl-2(3H)-benzothiazolylidene-1-[(1-ethyl-2(1H)-quinolinylidene)methyl]ethylidene;
(3-ethyl-2-oxazolidinylidene)ethylidene,

provided that the compound is not butyl-4-diphenyl-1,2-pyrazolidine-dione-3,5,

for preparing a pharmaceutical composition for treating viral diseases/infections.

2. Use of a compound according to claim 1
wherein
R^1 and R^2, which are similar of different, are chosen from the group comprising
hydrogen;
alkyl;
functionalised alkyl;
phenyl;

benzoyl which may be substituted in any position with one or more of the groups alkoxy, alkyl, fluorine, chlorine, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2, 3 or 4 and which may be substituted in position 5 with one of the groups fluorine and nitro;
2-thiazolyl which may be substituted in the 4 and/or 5 positions with one or two of the groups alkyl and phenyl;
R^3 and R^4, being similar or different, are chosen from the group comprising
hydrogen;
hydroxy;
fluorine;
alkyl;
functionalised alkyl;
hydroxyalkyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenylamino and diphenylamino wherein the phenyl groups may be similar or different;
aminoalkyl, (alkylamino)alkyl, (dialkylamino)alkyl, (phenylamino)alkyl and (diphenylamino)alkyl wherein the alkyl groups may be similar or different, and the phenyl groups may be similar or different;
(4-methylmorpholinyl)alkyl;
benzoyl which may be substituted in any position with one ore more of the groups alkoxy, alkyl, fluorine, chlorine, hydroxy and nitro;
pyridinyl which may be attached to the molecular core in position 2,3 or 4 and which may be substituted in position 5 with fluorine, chlorine, hydroxy or nitro;
alkanoyl;
oxoalkyl which may be substituted in any position with alkyl or phenyl;
phenylazo;

alkenyl;
phenyl(phenylimino)methyl;
phenyl;
2-furfuryl;
1H-indol-3-yl;
(1H-indol-3-yl)methyl;

and wherein R^3, R^4 is the methylidene group $=C(R^5)R^6$ wherein R^5 and R^6 being similar or different, are chosen form the group comprising
hydrogen;
hydroxy;
alkyl which may be completely or partially substituted with fluorine and chlorine;
alkenyl which may be substituted with one or more phenyl groups, and which alkenyl may be completely or partially substituted with deuterium, fluorine, chlorine, nitro, phenylthio, phenylsulphinyl and phenylsulphonyl;
phenyl;
amino, alkylamino and dialkylamino wherein the alkyl groups may be similar or different, or are 2-(diethylamino)ethyl;
phenymethyleneamin;
2-furanyl;
1H-indol-2-yl;
1H-indol-3-yl.

3. Use of a compound according to claim 1 for preparing a composition for treating HIV/AIDS.

4. Use of a compound according to claim 2 for preparing a composition for treating HIV/AIDS.

5. Use of a compound according to claim 1 for preparing a composition for treating measles.

6. Use of a compound according to claim 2 for preparing a composition for treating measles.

7. Use of a compound according to claim 1 wherein the compound is Oxyphenbutazone.

8. Use of Oxyphenbutazone for preparing a composition for treating HIV/AIDS.

9. Use of Oxyphenbutazone for preparing a composition for treating measles.

10. Use of a compound according to claim 7 for preparing a composition wherein Oxyphenbutazone is present in the composition in an amount suitable for delivering an effective dose of Oxyphenbutazone.

11. Use of a compound according to claim 10, wherein Oxyphenbutazone is present in the range of 0.0075 - 225 µmol/kg body weight.

12. Use of a compound according to claim 10, wherein Oxyphenbutazone is present in the range of 0.075 - 37.5 µmol/kg body weight.

13. Use of a compound according to claim 10, wherein Oxyphenbutazone is present in the range of 0.15 - 2.25 µmol/kg body weight.

14. Use of a compound according to claim 10 for preparing a composition suitable for being administered in 1 - 3 partial doses per day.

15. Use of a compound according to claim 1 for preparing a composition suitable for being administered

systemically, especially enterally, preferably parenterally, such as subcutananeously, intravenously, intradermally and orally, most preferably orally.

16. Use of a compound according to claim 10 for making a composition suitable for being administered systemically, especially enterally, preferably parenterally, such as subcutananeously, intravenously, intradermally and orally, most preferably orally.

17. Use of a compound according to claim 1 for preparing a composition including additionally an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor.

18. Use of a compound according to claim 10 for preparing a composition including additionally an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor.

19. A method of treating a viral infection wherein a compound according to claim 1 is administered to a subject in need of such a treatment.

20. A method according to claim 19 for treating HIV/AIDS.

21. A method according to claim 19 for treating measles.

22. A method according to claim 19 for treating a viral infection wherein a compound according to claim 1 is administered orally to a subject in need of such a treatment.

23.A method according to claim 20 for treating HIV/AIDS wherein a compound according to claim 1 is administered orally to a subject in need of such a treatment.

24.A method according to claim 21 for treating measles wherein a compound according to claim 1 is administered orally to a subject in need of such a treatment.

25.A method of treating a viral infection wherein a compound according to claim 10 is administered to a subject in need of such a treatment.

26.A method according to claim 25 for treating HIV/AIDS.

27.A method according to claim 25 for treating measles.

28.A method according to claim 25 for treating a viral infection wherein a compound according to claim 10 is administered orally to a subject in need of such a treatment.

29.A method according to claim 26 for treating HIV/AIDS wherein a compound according to claim 10 is administered orally to a subject in need of such a treatment.

30.A method according to claim 27 for treating measles wherein a compound according to claim 10 is administered orally to a subject in need of such a treatment.

31.A method according to claim 19 wherein a compound according to claim 1 in addition to an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor is aministered.

32. A method according to claim 25 wherein a compound according to claim 10 in addition to an inhibitor for inhibiting a reverse transcriptase enzyme and/or protease inhibitor and/or an integrase inhibitor is aministered.

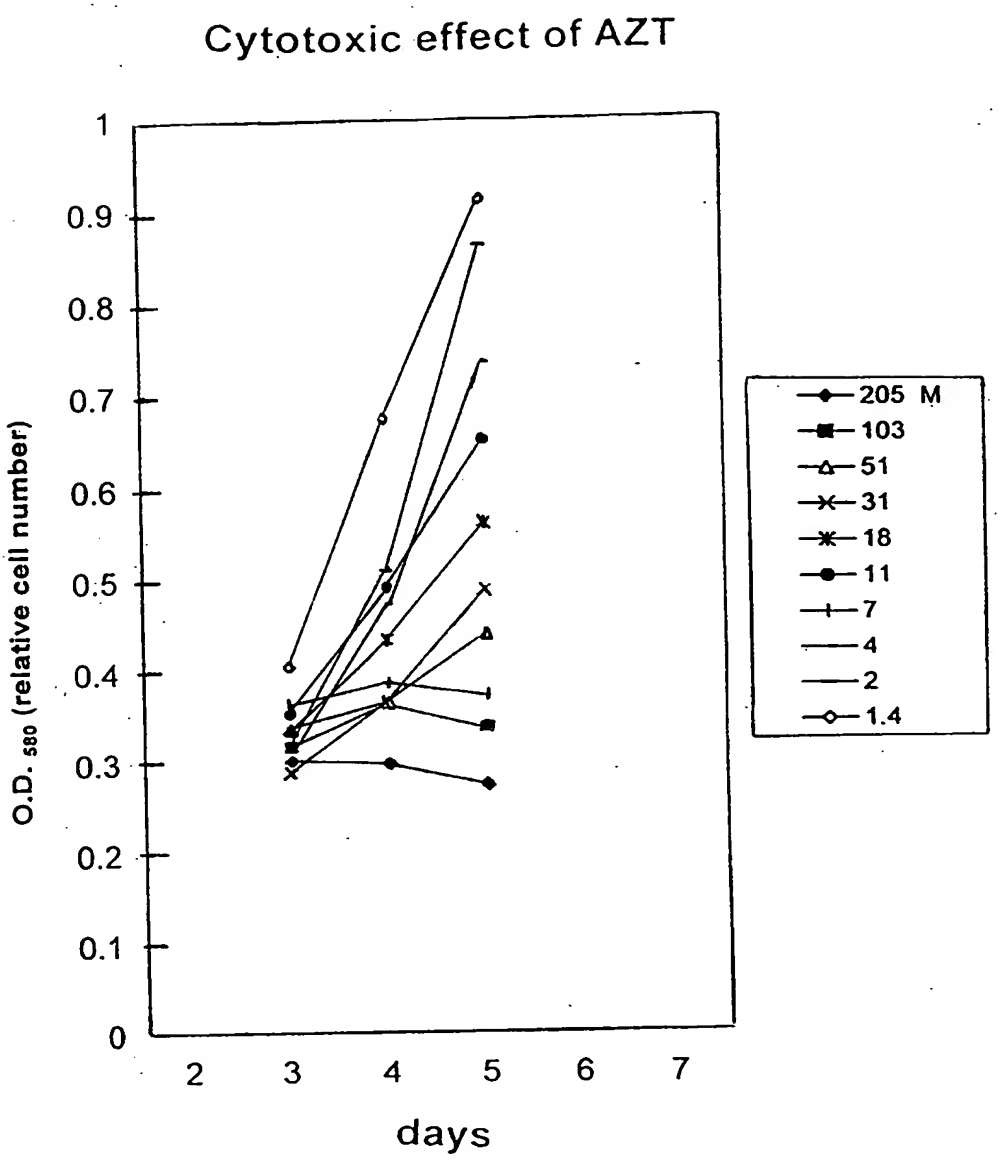

Cytotoxic effect of AZT
O.D. 580 (relative cell number)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2
3
4
5
6
7
days
205 M
103
51
31
18
11
7
4
2
1.4

Fig.1

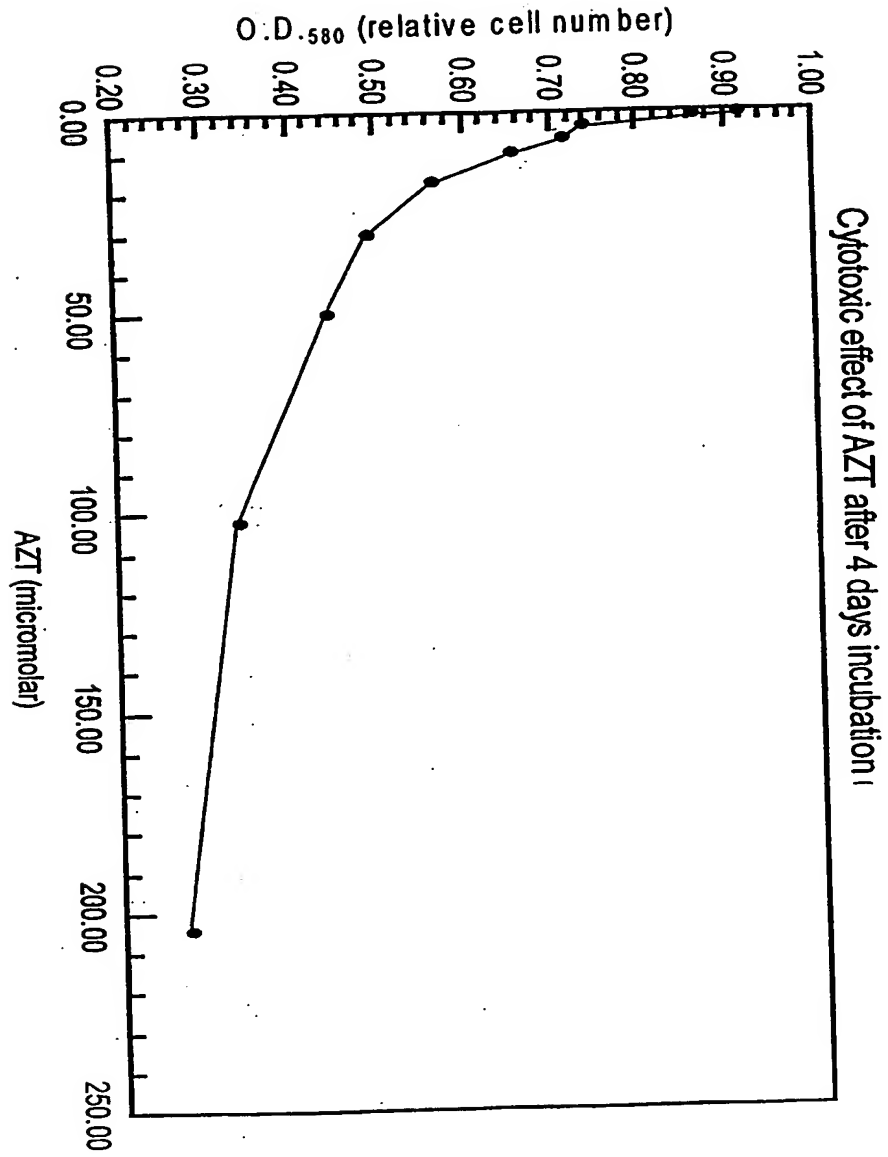

O.D.580 (relative cell number)
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
0.00
50.00
100.00
150.00
200.00
250.00
AZT (micromolar)
Cytotoxic effect of AZT after 4 days incubation

Fig.2

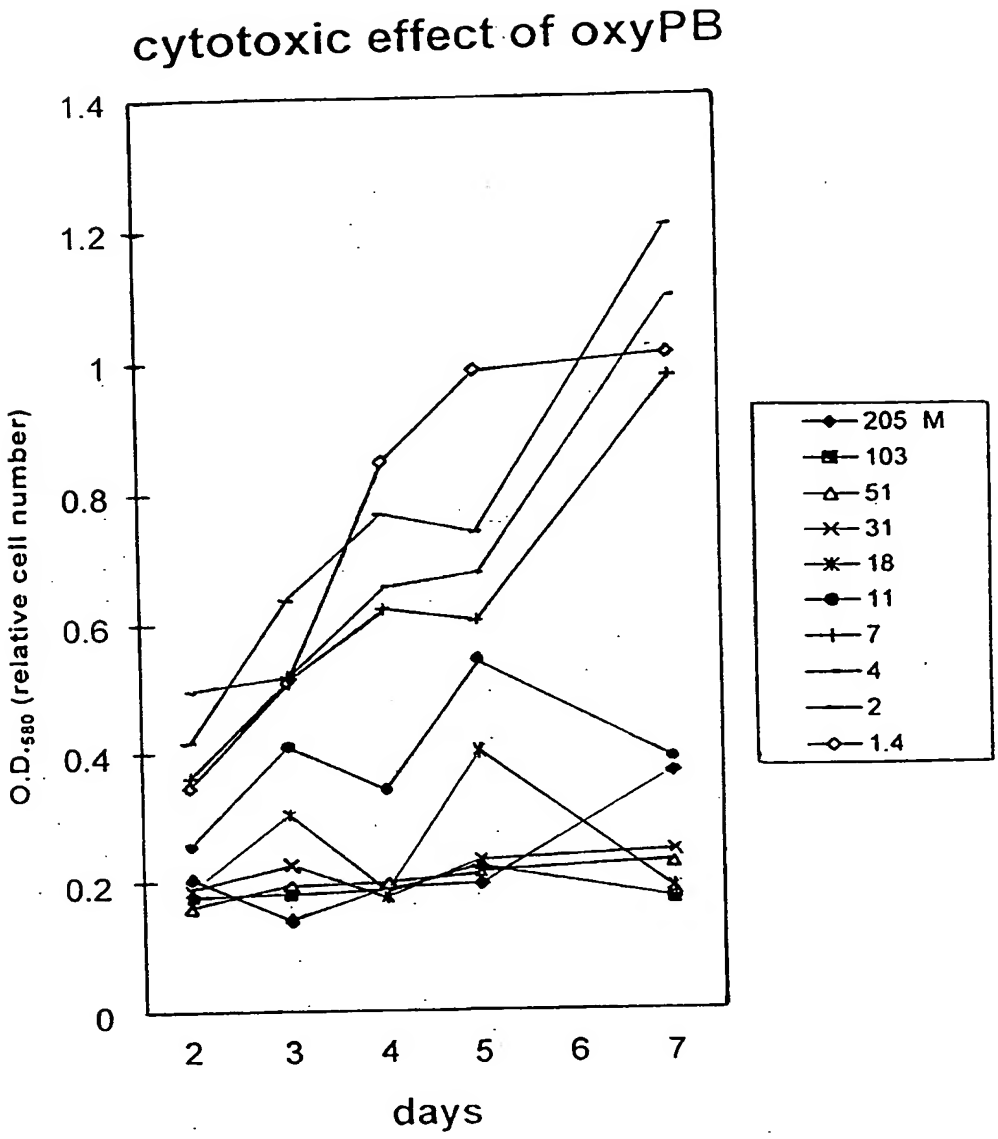

cytotoxic effect of oxyPB
1.4
1.2
1
0.8
0.6
0.4
0.2
0
O.D.580 (relative cell number)
2
3
4
5
6
7
days
205 M
103
51
31
18
11
7
4
2
1.4

Fig.3

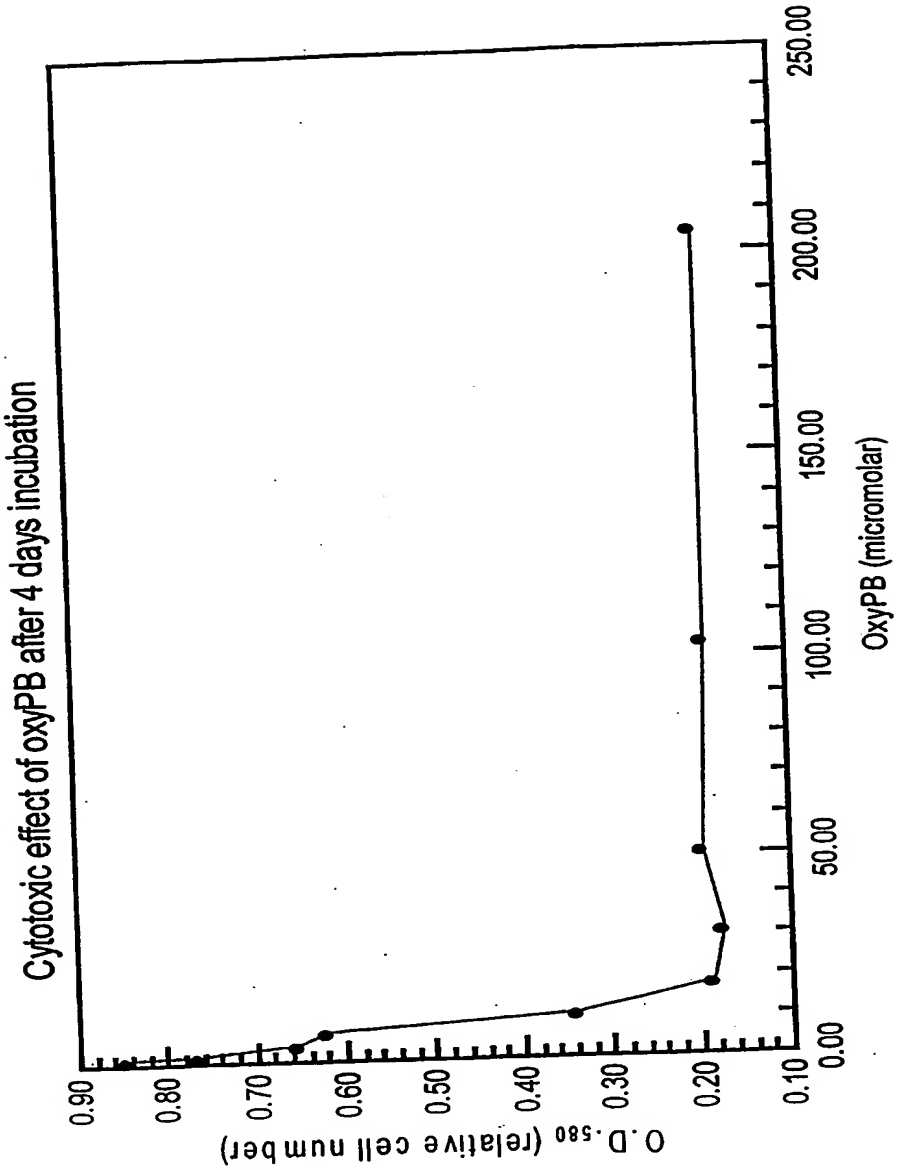

Cytotoxic effect of oxyPB after 4 days incubation
O.D.580 (relative cell number)
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
50.00
100.00
150.00
200.00
250.00
OxyPB (micromolar)

Fig.4

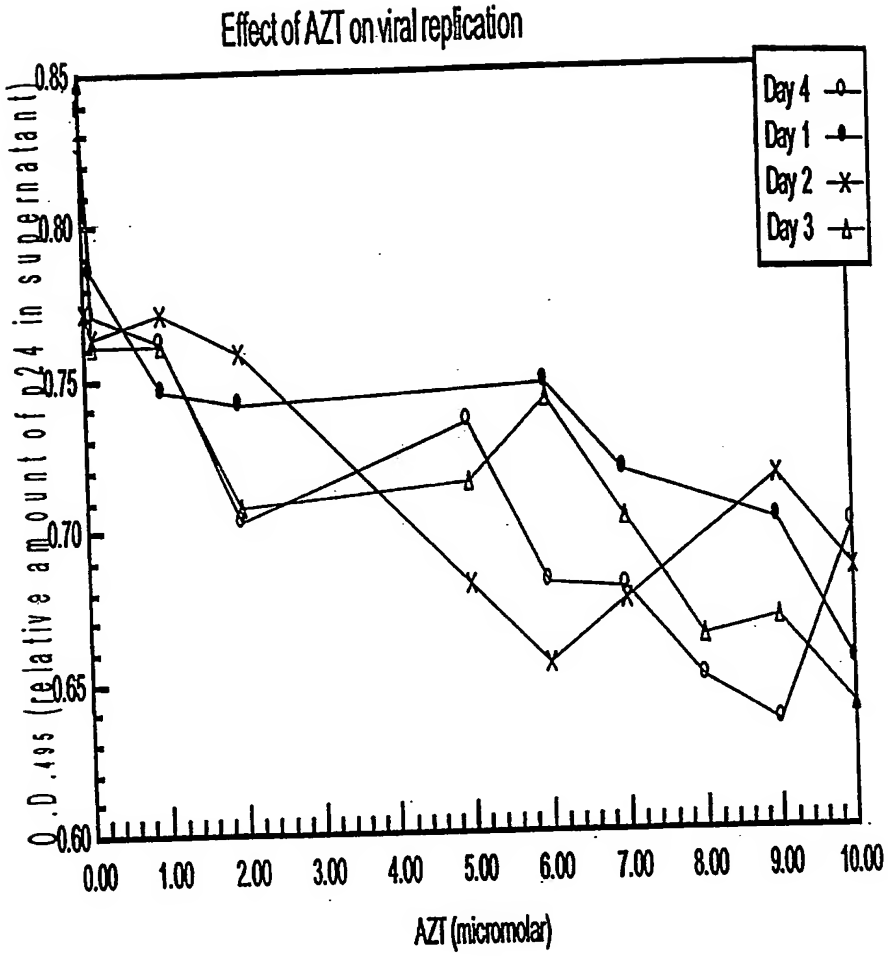

Effect of AZT on viral replication
Day 4
Day 1
Day 2
Day 3
O.D. 495 (relative amount of p24 in supernatant)
0.85
0.80
0.75
0.70
0.65
0.60
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
AZT (micromolar)

Fig.5

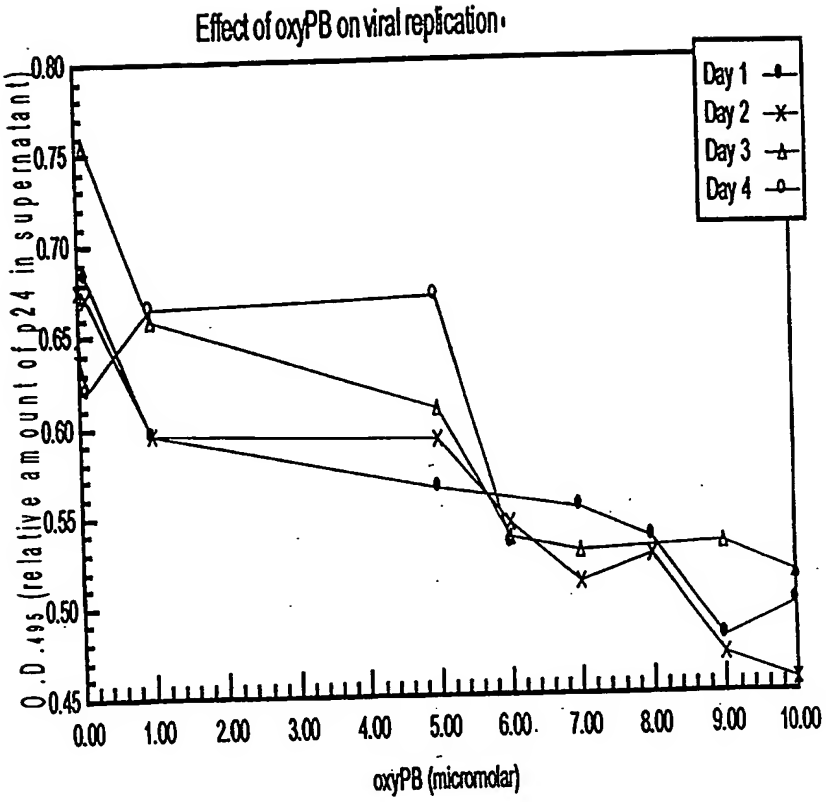
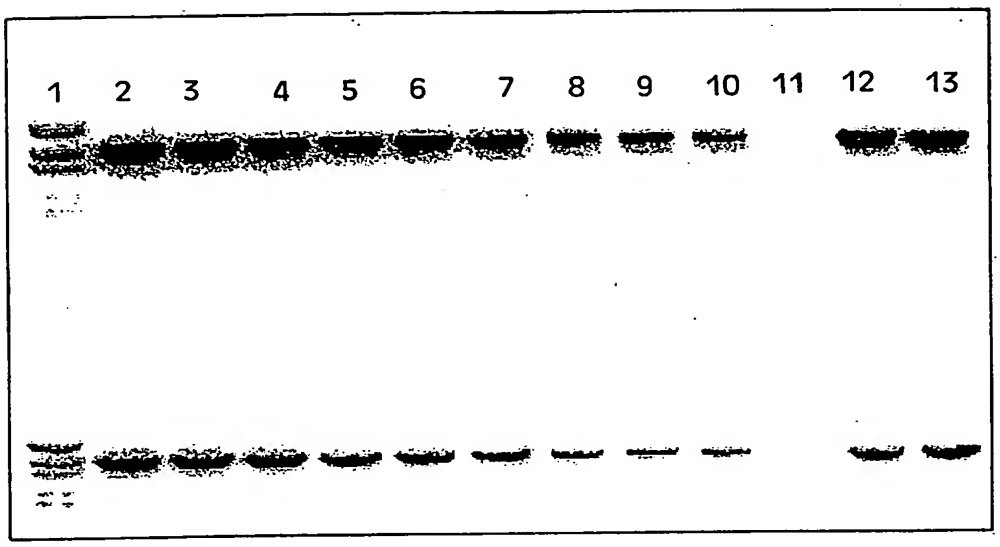

Effect of oxyPB on viral replication
Day 1
Day 2
Day 3
Day 4
O.D.495 (relative amount of p24 in supernatant)
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
oxyPB (micromolar)

Fig.6

Product of PVR analysed on agarose gel



1. pGEM
2. Positive control (no compound X, only water)
3. 5 µm X
4. 10 µm X
5. 20 µm X
6. 50 µm X
7. 100 µm X
8. 150 µm X
9. 200 µm X
10. 300 µm X
11. 500 µm X
12. Control containing only the solvent equal to the 500 µm sample
13. Control containing only the solvent equal to the 500 µm sample

Fig.7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 00/00086

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/4152, A61P 31/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0237796 A2 (RESEARCH DEVELOPMENT CORPORATION OF JAPAN ET AL), 23 Sept 1987 (23.09.87) --	1-32
X	US 4956377 A (MIESCH, JEAN-OLIVIER), 11 Sept 1990 (11.09.90) --	1-32
X	EP 0285730 A1 (MIESCH, JEAN-OLIVIER), 12 October 1988 (12.10.88) --	1-32

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 July 2000	17 -07- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO00/00086

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 19-32
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO00/00086

Claims 19-32 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.
PCT/NO 00/00086

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0237796 A2	23/09/87	DE 3785689 A	09/06/93
		JP 1888054 C	22/11/94
		JP 6010141 B	09/02/94
		JP 62190130 A	20/08/87
		US 4880742 A	14/11/89
US 4956377 A	11/09/90	EP 0285730 A,B	12/10/88
		SE 0285730 T3	
		FR 2593703 A	07/08/87
		JP 63267719 A	04/11/88
EP 0285730 A1	12/10/88	SE 0285730 T3	
		FR 2593703 A	07/08/87
		JP 63267719 A	04/11/88
		US 4956377 A	11/09/90